

**ATTORNEY DOCKET NO. 04156.0021U1**  
**Application No. 10/562,248**

**Amendments to the Claims**

The listing of claims will replace all prior versions, and listing, of claims in the application.

**Claims 1-31 (cancelled)**

32. (New) A semisterile culture method for producing a defined enzyme mixture, metabolite mixture, or combination thereof optimized for the fermentation of one or more target substrates, comprising

(a) contacting an inoculating mixed culture of microorganisms in a solid-phase bioreactor with one or more target substrates or a combination of one or more target substrates and one or more inducer substrates; and

(b) building up and keeping the mixed culture under an appropriate selection pressure by a suitable selection of the culturing parameters chosen from moisture content, pH value, temperature, oxygen availability, redox potential and nutrient composition and by a specific induction by the target substrate, inducer substrate, or combination thereof, inhibition with appropriate inhibitors for a defined culturing time, or a combination thereof.

33. (New) The method according to claim 32, wherein the inoculating mixed culture is obtainable by culturing a preculture of mixed microorganisms adapted to solid or liquid substrates cultivating on normal agar plates, other "solid-state" (SSF) cultures, or in any liquid cultures as an inoculating culture for the subsequent main SSF cultures.

34. (New) The method according to claim 33, wherein the preculture of mixed microorganisms is a mixed culture of fungi.

35. (New) The method according to claim 33, wherein the substrate is inductive.

36. (New) The method according to claim 33, wherein the inductive substrates, target substrates, or a combination thereof, are selected from all kinds of raw or waste materials of natural and non-natural industrial origin and their mixtures.

37. (New) The method according to claim 32, wherein the inoculating mixed culture

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- (a) is a culture which has run through an inductive preculture and one or more main cultures operated under selection pressure; or
- (b) is a culture which has run through an inductive preculture and one or more main cultures operated under selection pressure and is suitable to be employed for fermentation of the target substrate either directly or after preservation by freezing, lyophilization, or a combination thereof.

38. (New) The method according to claim 37, wherein the inductive substrates, target substrates, or a combination thereof, are selected from all kinds of raw or waste materials of natural and non-natural industrial origin and their mixtures.

39. (New) The method of claim 32, wherein the moisture content is used for controlling the selection pressure by the addition of water and its removal by means of temperature and suction.

40. (New) The method of claim 39, wherein the water activity is between 0.85 and 0.99.

41. (New) The method of claim 32, wherein at least two microorganisms are employed for producing mixed cultures and precultures of mixed microorganisms.

42. (New) The method of claim 41, wherein at least one microorganism is a fungi.

43. (New) The method of claim 42, wherein at least one microorganism is ascomycetes, deuteromycetes, white rot fungi, or brown rot fungi

44. (New) The method of claim 43, wherein at least one microorganism is of the fungi genera selected from the group consisting of Penicillium spec., Aspergillus spec., Trichoderma spec., Fusarium spec., Eurotium spec., Absidia spec., Neurospora spec., Mucor spec., Chaetomium sp., and Rhizopus sp. are employed as microorganisms.

45. (New) The method of claim 44, wherein at least one microorganism is a species of fungi selected from the group consisting of Penicillium chrysogenum, Eurotium amstelodami, Aspergillus niger, Aspergillus tubingensis, Trichoderma harzianum, Trichoderma atroviride, Trichoderma reesei, Fusarium oxysporum and Neurospora intermedia.

46. (New) The method of claim 43, wherein at least one microorganism is of the fungi genera selected from the group consisting of Trametes spec., Pleurotus spec., Phanerochaete spec., Nematoloma spec. and Agaricus spec.

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47. (New) The method of claim 41, wherein at least one microorganism is bacterium of the order actinomycetes.

48. (New) The method of claim 47, wherein at least one microorganism is a bacteria of the genus Streptomyces spec.

49. (New) The method of claim 32, wherein the method is performed in a continuous manner or in a step-wise manner with one or more process cycles.

50. (New) The method of claim 32, wherein the continuously produced enzyme/substrate/fungus mixtures are suitable applied as such.

51. (New) The method of claim 32, wherein the continuously produced enzyme/substrate/fungus mixtures are suitable after separation of the substrate/fungus mixture to obtain a liquid enzyme cocktail.

52. (New) The method of claim 32, wherein the continuously produced enzyme/substrate/fungus mixtures are suitable to be used for the saccharification of all kinds of natural polysaccharide substrates or for the degradation of vegetable, animal or microbial polymers.

53. (New) The method of claim 32, wherein the continuously produced enzyme/substrate/fungus mixtures are substituted by enzymes which are prepared by means of other methods or which are commercially available.

54. (New) The method of claim 32, wherein the continuously produced enzyme/substrate/fungus mixtures are suitable for fermentation under essentially anaerobic or anaerobic conditions.

55. (New) The method claim 32, wherein the mixed cultures are suitable for the continuous production of specific hydrolase cocktails, oxidoreductase cocktails, or a combination thereof, for processes target substrates.

56. (New) The method claim 55, wherein the hydrolase cocktails or oxidoreductase cocktails are suitable for use in the wood-processing industry, paper and pulp industries, textile industry, leather industry, animal-processing industry, detergent industry, fodder industry, food industry, waste water, exhaust air and soil purification, in the processing of residual materials, or in the processing of raw materials from naturally renewable resources.

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57. (New) The method of claim 32, wherein the enzyme mixture is a hydrolytic/oxidative enzyme cocktail and is suitable for the enzymatic extraction of sugar beet chips at least by means of a two-phase culture.

58. (New) The method of claim 32, wherein the enzyme mixture is a hydrolytic/oxidative enzyme cocktail and is suitable for the enzymatic extraction of vegetable, animal or microbial raw or waste materials before or after a chemical treatment, enzymatic treatment, microbial treatment, or combination thereof.

59. (New) The method of claim 32 for producing an enzyme mixture suitable for the enzymatic extraction of sugar beet chips or other polysaccharide containing material.

60. (New) The method of claim 59, wherein the inducer substrate is a rape extraction material.

61. (New) The method of claim 59, wherein the microorganisms are A. niger and A. tugibensis.

62. (New) The method of claim 59, wherein during the culture process Neurospora intermedia is added to the mixed culture and the water activity is reduced to about 0.96.

63. (New) The method of claim 32 for producing an enzyme mixture suitable for the enzymatic extraction of grass silage or other polysaccharide containing material.

64. (New) The method of claim 63, wherein the inducer substrate is a rape extraction material.

65. (New) The method of claim 63, wherein the microorganisms are A. niger, A. tugibensis and Neurospora intermedia.

66. (New) The method of claim 63, wherein during the culture process Trichoderma atroviridae and grass silage as substrate are added to the mixed culture and the water activity is raised to about 0.99.

67. (New) The method of claim 32 for producing an enzyme mixture suitable for the enzymatic extraction of corn silage or other polysaccharide containing material.

68. (New) The method of claim 67, wherein the inducer substrate is a rape extraction material.

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69. (New) The method of claim 67, wherein the microorganisms are *A. niger*, *A. tubigensis* and *Neurospora intermedia*.

70. (New) The method of claim 67, wherein during the culture process *Aspergillus oryzae* and corn silage as substrate are added to the culture and the water activity is raised to about 0.99.

71. (New) The culturing method of claim 32, wherein after optimum inoculation and selective process operation, the preinduced mixtures of microorganisms and enzyme mixtures are directly supplied to the downstream processes or first passed to a pre-hydrolysis container to effect a preliminary saccharification or a complete hydrolysis of the polysaccharides or other polymers.

72. (New) The culturing method of claim 32, wherein after optimum inoculation and selective process operation, the preinduced mixtures of microorganisms and enzyme mixtures are transferred to another solid state process operation in which the whole substrate which is to be fermented later is selectively utilized for producing enzymes and at least partially hydrolyzed.

73. (New) The method claim 32, wherein

- (a) said preinduced mixtures of microorganisms are mixtures of white rot fungi or mixtures of organisms which metabolize only low amounts of sugar at high enzyme forming rates; or
- (b) after optimum inoculation and selective process, the preinduced mixtures of microorganisms and enzyme mixtures, which were produced in a side stream in addition to the main solid-state process operation, are incorporated together with the main reaction into the subsequent fermentations by means of mixed populations of other microorganisms; or
- (c) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures are transferred to another solid state process operation in which the whole substrate is selectively utilized for producing enzymes for composting purposes; or
- (d) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures are transferred to

another solid state process operation in which the whole substrate is selectively utilized for producing enzymes for the degradation of xenobiotics; or

(e) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures are flowed through by liquid or gaseous induction substrates which are degraded or converted by the enzymes formed.

74. (New) The method of claim 32, wherein the solid-phase cultures are performed in screw reactor, drum reactor, tower reactors trickling film reactor, solid-state air-lift reactor, horizontal mixer, or vertical mixer.

75. (New) The method of claim 74, wherein the solid-phase cultures are performed according to the principle of screw conveying, pressure screw conveying, or conveying belt transport.

76. (New) The method of claim 74, wherein the solid-phase cultures are modified or in a cascade form.

77. (New) The method of claim 74, wherein the solid-phase cultures are performed

- (a) in a screw reactor either singly or arranged in a cascade form; or
- (b) in special solid-state air-lift reactors; or
- (c) as batch cultures, fed-batch cultures or continuously.

78. (New) The method of claim 32, further comprising conservation of the obtained mixed culture by decreasing the water activity during the fermentation process.

79. (New) The method of claim 78, wherein water activity is decreased by air flow through the substrate or by a final drying step.

80. (New) The method of claim 79, wherein the final drying step is in a fluidised bed or belt dryer.

81. (New) The method of claim 32, wherein a leaching of the produced enzyme mixture is carried out by moving or stirring it with water, buffer, detergent/water or detergent/buffer solutions for 30 min to 2 hours and wherein the obtained enzyme slurry is filtered and the filtrate is further used as a solvent for additional leaching cycles for receiving a highly concentrated enzyme slurry.

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82. (New) The method of claim 81, wherein the filtrate is used as a solvent for up to 10 additional leaching cycles.

83. (New) An enzyme mixture and a metabolite mixture obtainable according to the method of claim 32.

84. (New) The method according to claim 32, wherein the culturing is performed in a bioreactor comprising a fermentation module for the fermentation of substrates under selection pressure whereby the fermentation module comprises regulation means to adjust a fermentation environment, a feeding means being connected to the fermentation module to feed the substrate, an induction module for adding reagents to the fermentation media, a harvesting module comprising outlet means and a conveying means to convey the media from the fermentation module through the induction module to the harvesting module.